Mechanisms of action of antimicrobial drugs

Inhibition of cell wall synthesis

All β-lactam drugs are selective inhibitors of bacterial cell wall synthesis and therefore active against growing bacteria. The initial step in drug action consists of binding of the drug to cell receptors **(penicillin-binding proteins [PBPs])**. There are at least six different PBPs, some of which are transpeptidation enzymes. Different receptors have different affinities for a drug, and each may mediate a different effect. For example, attachment of penicillin to one PBP may result chiefly in abnormal elongation of the cell, but attachment to another PBP may lead to a defect in the periphery of the cell wall with resulting cell lysis. PBPs are under chromosomal control, and mutations may alter their number or their affinity for β-lactam drugs. After a β-lactam drug has attached to one or more receptors,
the transpeptidation reaction is inhibited, and peptidoglycan synthesis is blocked. The next step probably involves removal or inactivation of an inhibitor of autolytic enzymes in the cell wall. This activates the lytic enzyme and results in lysis if the environment is isotonic. In a markedly hypertonic environment, the microbes change to protoplasts or spheroplasts, covered only by the fragile cell membrane. In such cells, synthesis of proteins and nucleic acids may continue for some time. The inhibition of the transpeptidation enzymes by penicillins and cephalosporins may be attributable to a structural similarity of these drugs to acyl-d-alanyl-d-alanine. The transpeptidation reaction involves loss of a d-alanine from the pentapeptide.
The difference in susceptibility of Gram-positive and Gram-negative bacteria to various penicillins or cephalosporins probably depends on structural differences in their cell walls (eg, amount of peptidoglycan, presence of receptors and lipids, nature of cross-linking, and activity of autolytic enzymes) that determine penetration, binding, and activity of the drugs.
Resistance to penicillins may be determined by the organism's production of penicillin-destroying enzymes (β-lactamases). The **α-lactamases** open the β-lactam ring of penicillins and cephalosporins and abolish their antimicrobial activity. β-Lactamases have been described for many species of Gram-positive and Gram-negative bacteria. Some β-lactamases are plasmid mediated (eg, penicillinase of *Staphylococcus aureus*), and others are chromosomally mediated (eg, many species of Gram-negative bacteria).
There is one group of β-lactamases that is occasionally found in certain species of Gram-negative bacilli such as, *Klebsiella pneumoniae*. These enzymes are termed **extended-spectrum α-lactamases (ESBLs)** because they confer upon
the bacteria the additional ability to hydrolyze the β-lactam rings of cefotaxime, ceftazidime, or aztreonam.
The classification of β-lactamases is complex, based on the genetics, biochemical properties, and substrate affinity.

Clavulanic acid, sulbactam, and tazobactam are β-lactamase inhibitors that have

a high affinity for and irreversibly bind some β-lactamases (eg, penicillinase of *S. aureus*) but are not hydrolyzed by the β-lactamase. These inhibitors protect simultaneously present hydrolyzable penicillins (eg, ampicillin, amoxicillin, and piperacillin) from destruction.

Of most concern is the emergence of ***K. pneumoniae* carbapenemases (KPC)**, which are ESBLtype enzymes that confer resistance to third- and fourth-generation cephalosporins and carbapenems. This resistance mechanism is plasmid mediated and has spread nosocomially among many hospitals throughout the United States and other countries.

Although they were discovered in the mid-1960s, global spread of genes encoding metallo-β-lactamases has facilitated spread of these broad-range, inhibitor-resistant enzymes among many Gram-negative pathogens. This has ushered in an era of widespread dissemination of carbapenem-resistant Enterobacteriaceae possessing the VIM-type (Verona integron-encoded metallo-β-lactamase) and NDM-type (New Delhi metallo-β-lactamase) of these enzymes. VIMtype enzymes first appeared in *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, but have spread to Enterobacteriaceae.

Examples of agents acting by inhibition of cell wall synthesis are β-lactam drugs such as the penicillins, the cephalosporins, the carbapenems; the monobactam aztreonam; glycopeptide antibiotics such as vancomycin and teicoplanin;
and lipoglycopeptides such as oritavancin, telavancin, and dalbavancin. Several other drugs, including fosfomycin, bacitracin, cycloserine, and novobiocin, inhibit early steps in the biosynthesis of the peptidoglycan. Because the early stages of synthesis take place inside the cytoplasmic membrane, these drugs must penetrate the membrane to be effective.

Cephalosporins

Many cephalosporins are excreted mainly by the kidney and may accumulate and induce toxicity in patients with renal insufficiency.

Cephalosporins have been arranged into major groups, or “generations,”

First-Generation Cephalosporins

The first-generation cephalosporins are very active against Gram-positive cocci—except enterococci and MRSA—and are moderately active against some Gram-negative rods, primarily *E. coli*, *Proteus*, and *Klebsiella*. Anaerobic cocci are often sensitive, but *Bacteroides fragilis* is not. can be used to treat uncomplicated urinary tract infections and streptococcal pharyngitis. None of the first-generation drugs penetrate the CNS, and they are not drugs of first choice for any infection. Cefazolin is a choice for surgical prophylaxis because it gives the highest (90–120 μg/mL) levels with every 8-hour dosing.

Second-Generation Cephalosporins

The second-generation cephalosporins are a heterogeneous group. All are active against organisms covered by first-generation drugs but have extended coverage against Gram-negative rods, including *Klebsiella* and *Proteus* but not *P.*

aeruginosa. Some (not all) oral second-generation cephalosporins can be used to treat sinusitis and otitis media caused by *H. influenzae*, including β-lactamase-producing strains.

Cefoxitin and cefotetan used in mixed anaerobic infections, including peritonitis and pelvic inflammatory disease. However, resistance to these agents among the *B. fragilis* group has increased substantially.

Third-Generation Cephalosporins

The third-generation cephalosporins have decreased activity against Gram-positive cocci except for *S. pneumoniae*; enterococci are intrinsically resistant to cephalosporins and often produce superinfections during their use. When second-generation drugs tend to fail against *P. aeruginosa*, ceftazidime or cefoperazone may succeed. Thus, third-generation drugs are very useful in the management of hospital-acquired Gram-negative bacteremia. Ceftazidime may also be lifesaving in severe melioidosis (*Burkholderia pseudomallei* infection).

Another important distinguishing feature of several third-generation drugs, except cefoperazone, is the ability to reach the CNS and to appear in the spinal fluid in sufficient concentrations to treat meningitis caused by Gram-negative rods..

Fourth-Generation Cephalosporins

Cefepime is the only fourth-generation cephalosporin now in clinical use in the United States. It has enhanced activity against *Enterobacter* and *Citrobacter* species that are resistant to third-generation cephalosporins. Cefepime has activity comparable to that of ceftazidime against *P. aeruginosa*.

The activity against streptococci and methicillin-susceptible staphylococci is greater than that of ceftazidime and comparable with that of the other third-generation compounds.

Two agents, ceftaroline and ceftobiprole, have activity against MRSA.

However, these agents do not have good activity against *P. aeruginosa*, *Acinetobacter* species, or ESBL-producing Enterobacteriaceae.

Inhibition/alteration of cell membrane function

The cytoplasmic membrane of bacteria and fungi has a structure different from that of animal cells and can be more readily disrupted by certain agents.

Detergents, which contain lipophilic and hydrophilic groups, disrupt cytoplasmic membranes and kill the cell. The **polymyxins**, consists of detergent - like cyclic peptides that selectively damage membranes containing phosphatidylethanolamine, a major component of bacterial membranes. **Daptomycin** is a cyclic 13-member lipopeptide antibiotic that is rapidly bactericidal by binding to the cell membrane in a calcium ion-dependent manner, causing depolarization of bacterial membrane potential. This leads to intracellular potassium release that causes cell death. Currently, this agent is approved for use in the treatment of *S. aureus* bloodstream infections and skin and soft tissue infections caused by Gram-positive bacteria, particularly organisms that are highly resistant to β-lactam agents and vancomycin.

Inhibition of protein synthesis

It is established that macrolides, lincosamides, tetracyclines, glycylcyclines, aminoglycosides, and chloramphenicol can inhibit protein synthesis in bacteria. The precise mechanisms of action differ among these classes of drugs.

In normal microbial protein synthesis, the mRNA message is simultaneously "read" by several ribosomes that are strung out along the mRNA strand. These are called **polysomes**.

Aminoglycosides

The mode of action of streptomycin has been studied far more intensively than that of other aminoglycosides, but all act similarly. The first step is the attachment of the aminoglycoside to a specific receptor protein (P 12 in the case of streptomycin) on the 30S subunit of the microbial ribosome.

Second, the aminoglycoside blocks the normal activity of the "initiation complex" of peptide formation (mRNA + formyl methionine + tRNA). Third, the mRNA message is misread on the "recognition region" of the ribosome; consequently, the wrong amino acid is inserted into the peptide, resulting in a nonfunctional protein. Fourth, aminoglycoside attachment results in the breakup of polysomes and their separation into **monosomes** incapable of protein synthesis.

Chromosomal resistance of microbes to aminoglycosides principally depends on the lack of a specific protein receptor (modification of the target site caused by mutations) on the 30S subunit of the ribosome. Plasmid-dependent resistance to aminoglycosides depends on the production by the microorganism of adenylating, phosphorylating, or acetylating enzymes that destroy the drugs (most common mechanism).

A third type of resistance consists of a "permeability defect," an outer membrane change that reduces active transport of the aminoglycoside into the cell so the drug cannot reach the ribosome. Often this is plasmid mediated.

Macrolides and Ketolides (erythromycin, azithromycin, clarithromycin, fidaxomicin, and the ketolide telithromycin) bind to the 50S subunit of the ribosome, and the binding site is domain V of the 23S rRNA. They may interfere with formation of initiation complexes for peptide chain synthesis or may interfere with aminoacyl translocation reactions. Some macrolide-resistant bacteria lack the proper receptor on the ribosome (through methylation of the 23S rRNA target site). The *erm* (erythromycin ribosome methylation) genes that encode this mechanism may be under plasmid or chromosomal control.

Tetracyclines

Tetracyclines bind reversibly to the 30S subunit of microbial ribosomes. They inhibit protein synthesis by blocking the attachment of charged aminoacyl-tRNA. Thus, they prevent introduction of new amino acids to the nascent peptide chain. The action is usually inhibitory and reversible upon withdrawal of the drug. Resistance to tetracyclines occurs by multiple mechanisms—efflux, ribosomal protection proteins, and chemical modification, among others.

The first two are the most important and occur as follows:
Efflux pumps, located in the bacterial cell cytoplasmic membrane, are responsible for pumping the drug out of the cell. *Tet* gene products are responsible for protecting the ribosome, likely through mechanisms that induce conformational changes. These conformational changes either prevent binding of the tetracyclines or cause their dissociation from the ribosome.

Glycylcyclines

The glycylcyclines are synthetic analogues of the tetracyclines. The agent that is available for use in the United States and Europe is tigecycline, a derivative of minocycline.

Chloramphenicol

Chloramphenicol binds to the 50S subunit of the 70S bacterial ribosome. It interferes with the binding of new amino acids to the nascent peptide chain, largely because chloramphenicol inhibits peptidyl transferase. Chloramphenicol is mainly bacteriostatic, and growth of microorganisms resumes when the drug is withdrawn.
Microorganisms resistant to chloramphenicol usually produce the chloramphenicol acetyltransferases, which destroys drug activity. The roduction of this enzyme is usually under the control of plasmid-mediated resistance genes called *cat* genes. Other mechanisms of resistance include efflux pumps and decreased membrane permeability.

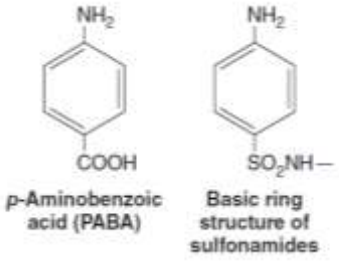
Oxazolidinones

The oxazolidinones possess a unique mechanism of inhibition of protein synthesis primarily in Gram-positive bacteria. These compounds interfere with translation by inhibiting the formation of *N*-formyl-methionyl-tRNA, the initiation complex at the 23S ribosome. Linezolid was the first agent to become commercially available and it has seen widespread usage in the treatment of a variety of serious Gram-positive infections including those caused by vancomycin-resistant enterococci and even mycobacterial infections.

Inhibition of nucleic acid synthesis

Examples of drugs acting by inhibition of nucleic acid synthesis are quinolones, pyrimethamine, rifampin, sulfonamides, trimethoprim, and trimetrexate.
Rifampin inhibits bacterial growth by binding strongly to the DNA-dependent RNA polymerase of bacteria. Thus, it inhibits bacterial RNA synthesis. Rifampin resistance results from a change in RNA polymerase because of a chromosomal mutation that occurs with high frequency.
All quinolones and fluoroquinolones inhibit microbial DNA synthesis by blocking DNA gyrases, topoisomerase enzymes that play key roles in DNA replication and repair.
For many microorganisms, *p*-aminobenzoic acid (PABA) is an essential metabolite. The specific mode of action of PABA involves an adenosine triphosphate (ATP)-dependent condensation of a pteridine with PABA to yield dihydropteroic acid, which is subsequently converted to folic acid.

PABA is involved in the synthesis of folic acid, an important precursor to the synthesis of nucleic acids. Sulfonamides are structural analogs of PABA and inhibit dihydropteroate synthetase.



Sulfonamides can enter into the reaction in place of PABA and compete for the active center of the enzyme. As a result, nonfunctional analogs of folic acid are formed, preventing further growth of the bacterial cell. The inhibiting action of sulfonamides on bacterial growth can be counteracted by an excess of PABA in the environment (competitive inhibition). Animal cells cannot synthesize folic acid and must depend on exogenous sources. Some bacteria, similar to animal cells, are not inhibited by sulfonamides. Many other bacteria, however, synthesize folic acid, as mentioned earlier, and consequently are susceptible to action by sulfonamides.

Trimethoprim (3,4,5-trimethoxybenzylpyrimidine) inhibits dihydrofolic acid reductase 50,000 times more efficiently in bacteria than in mammalian cells. This enzyme reduces dihydrofolic to tetrahydrofolic acid, a stage in the sequence leading to the synthesis of purines and ultimately of DNA.

Sulfonamides and trimethoprim each can be used alone to inhibit bacterial growth. If used together, they produce sequential blocking, resulting in a marked enhancement (synergism) of activity. Such mixtures of sulfonamide (five parts) plus trimethoprim (one part) have been used in the treatment of pneumocystis pneumonia, malaria, shigella enteritis, systemic salmonella infections, urinary tract infections, and many others.

Pyrimethamine also inhibits dihydrofolate reductase, but it is more active against the enzyme in mammalian cells and therefore is more toxic than trimethoprim. Pyrimethamine plus sulfonamide or clindamycin is the current treatment of choice in toxoplasmosis and some other protozoal infections.

Resistance to antimicrobial drugs

There are many different mechanisms by which microorganisms might exhibit resistance to drugs.

1. Microorganisms produce enzymes that destroy the active drug. ***Examples:*** Staphylococci resistant to penicillin G produce a β-lactamase that destroys the drug. Other β-lactamases are produced by Gram-negative rods. Gram-negative bacteria resistant to aminoglycosides (by virtue of a plasmid) produce adenylating, phosphorylating, or acetylating enzymes that destroy the drug.

2. Microorganisms change their permeability to the drug. ***Examples:*** Tetracyclines accumulate in susceptible bacteria but not in resistant bacteria. Resistance to polymyxins is also associated with a change in permeability to the drugs. Streptococci have a natural permeability barrier to aminoglycosides. This can be partly overcome by the simultaneous presence of a cell wall-active drug such as penicillin. Resistance to amikacin and to some other aminoglycosides may depend on a lack of permeability to the drugs caused by an outer membrane change that impairs active transport into the cell.
3. Microorganisms develop an altered structural target for the drug ***Examples:*** Erythromycin-resistant organisms have an altered receptor on the 50S subunit of the ribosome, resulting from methylation of a 23S ribosomal RNA. Resistance to some penicillins and cephalosporins may be a function of the loss or alteration of PBPs. Penicillin resistance in *Streptococcus pneumoniae* and enterococci is attributable to altered PBPs.
4. Microorganisms develop an altered metabolic pathway that bypasses the reaction inhibited by the drug. ***Example:*** Some sulfonamide-resistant bacteria do not require extracellular PABA but, similar to mammalian cells, can use preformed folic acid.
5. Microorganisms develop an altered enzyme that can still perform its metabolic function but is much less affected by the drug. ***Example:*** In trimethoprim-resistant bacteria, the dihydrofolic acid reductase is inhibited far less efficiently than in trimethoprim-susceptible bacteria.
6. Microorganisms can develop efflux pumps that transport the antibiotics out of the cell. Many Gram-positive and especially Gram-negative organisms have developed this mechanism for tetracyclines (common), macrolides, fluoroquinolones, and even β-lactam agents.

Origin of drug resistance

Nongenetic Origin of Drug Resistance

Active replication of bacteria is required for most antibacterial drug actions. Consequently, microorganisms that are metabolically inactive (nonmultiplying) may be phenotypically resistant to drugs. However, their offspring are fully susceptible. ***Example:*** *Mycobacteria* often survive in tissues for many years after infection yet are restrained by the host's defenses and do not multiply. Such "persisting" organisms are resistant to treatment and cannot be eradicated by drugs. Yet if they start to multiply (eg, after the suppression of cellular immunity in the patient), they are fully susceptible to the same drugs. Microorganisms may lose the specific target structure for a drug for several generations and thus be resistant. ***Example:*** Penicillin-susceptible organisms may change to cell wall deficient L forms during penicillin administration. Lacking cell walls, they are resistant to cell wall-inhibitor drugs (penicillins, cephalosporins) and may remain so for several generations. When these organisms revert to their bacterial parent forms by resuming cell wall production, they are again susceptible to penicillin.

Microorganisms may infect the host at sites where antimicrobials are excluded or are not active. ***Examples:*** Aminoglycosides such as gentamicin are not effective in treating *Salmonella* enteric fevers because the salmonellae are intracellular and the aminoglycosides do not enter the cells. Similarly, only drugs that enter cells are effective in treating Legionnaires' disease because of the intracellular location of *Legionella pneumophila*.

Genetic Origin of Drug Resistance

Most drug-resistant microbes emerge as a result of genetic change and subsequent selection processes by antimicrobial drugs.

A. Chromosomal Resistance

This develops as a result of spontaneous mutation in a locus that controls susceptibility to a given antimicrobial drug. The presence of the antimicrobial drug serves as a selection mechanism to suppress susceptible organisms and favor the growth of drug-resistant mutants. Spontaneous mutation occurs with a frequency of 10^{-12}–10^{-7} and thus is an infrequent cause of the emergence of clinical drug resistance in a given patient.

However, chromosomal mutants resistant to rifampin occur with high frequency (~10^{-7}–10^{5}). Consequently, treatment of bacterial infections with rifampin as the sole drug often fails. Chromosomal mutants are most commonly resistant by a change in a structural receptor for a drug. Thus, the P 12 protein on the 30S subunit of the bacterial ribosome serves as a receptor for streptomycin attachment. Mutation in the gene controlling that structural protein results in streptomycin resistance. Mutation can also result in the loss of PBPs, making such mutants resistant to β-lactam drugs.

B. Extrachromosomal Resistance

Bacteria often contain extrachromosomal genetic elements called plasmids.

Some plasmids carry genes for resistance to one—and often several—antimicrobial drugs. Plasmid genes for antimicrobial resistance often control the formation of enzymes capable of destroying the antimicrobial drugs. Thus, plasmids determine resistance to penicillins and cephalosporins by carrying genes for the formation of β-. Plasmids code for enzymes that acetylate, adenylate, or phosphorylate various aminoglycosides; for enzymes that determine the active transport of tetracyclines across the cell membrane; and for others. Genetic material and plasmids can be transferred by transduction, transformation, and conjugation.

Limitation of drug resistance

Emergence of drug resistance in infections may be minimized in the following ways:

(1) by maintaining sufficiently high levels of the drug in the tissues to inhibit both the original population and first-step mutants;

(2) by simultaneously administering two drugs that do not give cross-resistance, each of which delays the emergence of mutants resistant to the other drug (eg, rifampin and isoniazid [INH] in the treatment of tuberculosis); and

(3) by avoiding exposure of microorganisms to a particularly valuable drug by limiting its use, especially in hospitals.

Mycobacterium tuberculosis

Primary drug resistance in *M. tuberculosis* occurs in about 10% of isolates and most commonly is to INH or streptomycin. Resistance to rifampin or ethambutol is less common. INH and rifampin are the primary drugs used in most standard treatment regimens; other first-line drugs are pyrazinamide and ethambutol. Resistance to INH and rifampin is considered multiple drug resistance. Poor compliance with drug treatment is a major factor in the development of drug resistance during therapy. Control of MDR-TB is a significant worldwide problem. More recently, emergence of extensively drug-resistant TB (XDR-TB) presents a significant challenge to global tuberculosis control. In addition to resistance to INH and rifampin, these organisms are also resistant to quinolones and injectable drugs, such as aminoglycosides or capreomycin (second-line agents).

Antimicrobial drugs used in combination

Indications

Possible reasons for using two or more antimicrobials simultaneously instead of a single drug are as follows:

1. To give prompt treatment in desperately ill patients suspected of having serious microbial infections. A good guess, usually based on available antibiogram data, about the most probable two or three pathogens, is made, and drugs are aimed at those organisms. Before such treatment is started, it is essential that adequate specimens be obtained for identifying the etiologic agent in the laboratory.

Suspected Gram-negative or staphylococcal sepsis in immunocompromised patients and bacterial meningitis in children are the foremost indications in this category.

2. To delay the emergence of microbial mutants resistant to one drug in chronic infections by the use of a second or third non-cross-reacting drug. The most prominent example is treatment for active tuberculosis.

3. To treat mixed infections, particularly those after massive trauma or those involving vascular structures. Each drug is aimed at an important pathogenic microorganism.

4. To achieve bactericidal synergism or to provide bactericidal action. In a few infections, such as enterococcal sepsis, a combination of drugs is more likely to eradicate the infection than either drug used alone. Such synergism is only partially predictable, and a given drug pair may be synergistic for only a single microbial strain. Occasionally, simultaneous use of two drugs permits significant reduction in dose and thus avoids toxicity but still provides satisfactory antimicrobial action.

Disadvantages

The following disadvantages of using antimicrobial drugs in combinations must always be considered:

1. The physician may believe that because several drugs are already being given, everything possible has been done for the patient, leading to relaxation of the effort to establish a specific diagnosis. It may also give a false sense of security.
2. The more drugs that are administered, the greater the chance for drug reactions to occur or for the patient to become sensitized to drugs.
3. The cost is unnecessarily high.
4. Antimicrobial combinations usually accomplish no more than an effective single drug.
5. Very rarely, one drug may antagonize a second drug given Simultaneously.

Antimicrobial synergism can occur in several types of situations.

1. Two drugs may sequentially block a microbial metabolic pathway. Sulfonamides inhibit the use of extracellular PABA by some microbes for the synthesis of folic acid. Trimethoprim or pyrimethamine inhibits the next metabolic step, the reduction of dihydro- to tetrahydrofolic acid.
2. A drug such as a cell wall inhibitor (a penicillin or cephalosporin) may enhance the entry of an aminoglycoside into bacteria and thus produce synergistic effects. Penicillins enhance the uptake of gentamicin or streptomycin by enterococci. Thus, ampicillin plus gentamicin may be essential for the eradication of *Enterococcus faecalis*,particularly in endocarditis.
3. One drug may prevent the inactivation of a second drug by microbial enzymes. Thus, inhibitors of β-lactamase (eg, clavulanic acid, sulbactam, and tazobactam) can protect amoxicillin, piperacillin, and other β-lactam agents from inactivation by β-lactamases.

Antimicrobial antagonism

Antagonism resulting in higher morbidity and mortality rates has been most clearly demonstrated in bacterial meningitis. It occurred when a bacteriostatic drug (which inhibited protein synthesis in bacteria) such as chloramphenicol or tetracycline was given with a bactericidal drug such as a penicillin or an aminoglycoside. Antagonism occurred mainly if the bacteriostatic drug reached the site of infection before the bactericidal drug, if the killing of bacteria was essential for cure, and if only minimal effective doses of either drug in the pair were present.

Antimicrobial chemoprophylaxis

Anti-infective chemoprophylaxis implies the administration of antimicrobial drugs to prevent infection. In a broader sense, it also includes the use of antimicrobial drugs soon after the acquisition of pathogenic microorganisms (eg, after compound fracture) but before the development of signs of infection.

Useful chemoprophylaxis is limited to the action of a specific drug on a specific organism.